Improved Electrical Coupling in Uterine Smooth Muscle Is Associated with Increased Numbers of Gap Junctions at Parturition

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ABSTRACT We have studied some passive electrical properties of uterine smooth muscle to determine whether a change in electrical parameters accompanies gap junction formation at delivery. The length constant of the longitudinal myometrium increased from 2.6 ± 0.8 mm ($\bar{X}$ ± SD) before term to 3.7 ± 1 mm in tissues from delivering animals. The basis of the change was a 33% decrease in internal resistance and a 46% increase in membrane resistance. Axial current flow in an electrical syncytium such as myometrium is impeded by the cytoplasm of individual cells plus the junctions between cells. Measurement of the longitudinal impedance indicated that the specific resistance of the myoplasmic component was constant at 319 ± 113 Ω·cm before term and 340 ± 93 Ω·cm at delivery. However, a decrease in junctional resistance was apparent from 323 ± 161 Ω·cm to 134 ± 64 Ω·cm at delivery. 1.5–2 d after delivery, the junctional resistance was increased, as was the myoplasmic resistance. Thin-section electron microscopy of some of the same muscle samples showed that gap junctions were present in significantly greater numbers in the delivering tissues. Therefore, our results support the hypothesis that gap junction formation at delivery is associated with improved electrical coupling of uterine smooth muscle.

INTRODUCTION

The development of synchronous muscular activity is characteristic of uterine smooth muscle at the end of pregnancy. Throughout gestation the myometrium is relatively quiescent and different areas behave as if they were functionally independent. Shortly before the onset of delivery, the electrical and mechanical activity of different parts of the uterus become, and then remain, synchronous (Csapo, 1962; Csapo and Takeda, 1965; Fuchs, 1969; for review, Liggins, 1979). The coordinated contractile activity of the smooth muscle is effective in delivering the fetuses.

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J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/82/09/0353/23 $1.00 353
Volume 80 September 1982 353–375

Uterine muscle, like most smooth muscle types, is a functional electrical, though not anatomical, syncytium (Abe, 1971; Tomita, 1975). It consists of bundles of small smooth muscle cells in series and in parallel, each cell 300–600 μm long and 5–10 μm wide at term (Csapo, 1962; Finn and Porter, 1975). Uterine electrical activity (and the resulting contractile activity) is initiated at mobile but discrete pacemaker regions (Lodge and Sproat, 1981) and spreads from one part of the tissue to the next in much the same way as within a single cell. Therefore, electrical interaction between cells, usually taken to imply coupling by low-resistance pathways of some sort, is necessary for coordinated activity of the myometrium.

Earlier studies have revealed that gap junctions between uterine smooth muscle cells were absent or present only in very low frequency during pregnancy. Their frequency was dramatically increased immediately before, during, and for a short time after parturition in the rat (Garfield et al., 1977, 1978). Gap junctions are generally thought to be the structures that mediate ionic and metabolic coupling between many cell types (Bennett and Goodenough, 1978; Loewenstein, 1981). In some cases however, the morphological basis of coupling in a syncytium is uncertain, for electrical coupling can apparently exist in the absence of ultrastructurally detectable gap junctions (Daniel et al., 1976; Williams and DeHaan, 1981). We have proposed that gap junction formation might be instrumental in improving the coupling between smooth muscle cells at the time of delivery (Garfield et al., 1977, 1978). The improved electrical communication among cells could facilitate synchronous excitation of a large number of fibers and permit the evolution of effective, coordinated uterine activity, resulting in termination of pregnancy.

In this paper we examine the question of whether improved electrical coupling is evident at a time when gap junctions are present in large numbers. The small size of smooth muscle cells and the complex three-dimensional geometry of the intact, syncytial tissues preclude the direct measurement of coupling between adjacent cells. However, measurements of passive cable properties and the specific resistance of the muscle can yield indirect estimates of ionic coupling between smooth muscle cells (Abe and Tomita, 1968; Tomita, 1969, 1975). Two independent methods are used to evaluate the internal resistance of myometrium and relate the improved coupling at parturition to increased numbers of gap junctions in the muscle.

METHODS

Experimental Preparation

Wistar rats at various stages of pregnancy were used in this study. The timing of pregnancy was determined by examination of vaginal smears; the presence of sperm the morning after a male rat was placed in the cage with a female indicated day one of pregnancy. Animals were stunned and then killed by cervical dislocation. Uterine horns were excised and placed in Krebs solution (20–22°C) in a petri dish lined with Sylguard (Dow Corning Corp., Midland, MI). The composition of the Krebs solution was (in mM): 115.5 NaCl; 4.6 KCl; 2.5 $CaCl_2$; 1.2 $MgSO_4$; 1.2 NaH_2PO_4; 22.1

$NaHCO_3$; 11.1 glucose. The Krebs solution had been equilibrated with 95% O_2–5% CO_2 and had a pH of 7.4. Each horn was pinned at its in vivo length and a longitudinal cut was made along the mesometrial attachment. The fetal contents were removed and the uterine tissues were laid flat, serosal side up, pinned at the original length. Under a binocular microscope, longitudinal muscle pieces were dissected from the antimesometrial area alongside the linea uteri. Successful dissections yielded uninterrupted bundles of longitudinal muscle.

Cable Analysis

Tissues 2 cm long and 1–2 mm wide were transferred to a partitioned organ bath of a design described by Abe and Tomita (1968) and maintained at their in vivo length. We arranged the specimens so that 1 cm of the ovarian end of the myometrium was located between the stimulating electrodes and the other 1 cm protruded into the recording chamber. Superfusion with Krebs solution at 30°C occurred at a rate of 1–2 ml/min.

The membrane responses of superficial smooth muscle cells were recorded with intracellular microelectrodes. Electrodes were filled with filtered 3 M KCl and had resistances of 30–50 MΩ. Microelectrodes were connected to the probe of an electrometer (WP Instruments, Inc., New Haven, CT) and the voltage signal was displayed on one channel of a dual-beam storage oscilloscope (D-13; Tektronix Inc., Beaverton, OR). The voltage gradient applied to the tissues with external Ag-AgCl electrodes was measured with two platinum wires in the stimulating chamber. Film records were obtained by directly photographing the oscilloscope screen.

The length constant (λ) was determined as the distance for decay of the electrotonic potential to e^{-1} when plotted as $\log_{10}$ voltage vs. distance. To obtain the membrane time constant (τ_m), the time to reach 50% of the steady state potential was plotted against distance from the nearest electrode. A single value of λ and τ_m was determined from multiple impalements of each tissue and mean values were determined for each cable parameter.

Measurement of the Longitudinal Impedance

We measured the longitudinal impedance of uterine smooth muscle with a technique essentially the same as that described by Ohba et al. (1976). Preliminary experiments performed on guinea pig taenia coli muscle revealed that the myoplasmic resistance was 232 ± 69 Ω·cm and the junctional resistance was 419 ± 119 Ω·cm ($n = 11$). Thus we confirmed the values of 214 Ω·cm for myoplasmic resistance and 372 Ω·cm for junctional resistance reported by Ohba et al. (1976). Tissues were weighed then mounted at their in vivo length in the horizontal part of a T-tube cut in a Plexiglas block. Pt black electrodes were placed at both ends of the horizontal tube and made contact with the tissue. The impedance of the muscle between the electrodes was determined by measuring the current produced by a sinusoidal voltage applied across the chamber (4800A vector impedance meter; Hewlett-Packard Co., Palo Alto, CA). Tissues responses were independent of variations in current magnitude ranging from 3×10^{-7} to 3×10^{-6} A. The phase shift between the current and voltage was also recorded.

After being mounted in the experimental chamber, the tissues were superfused with Krebs solution through the vertical tube and allowed to recover for 45 min at 20–22°C. At the beginning of each experiment Krebs was replaced with isosmotic sucrose (92 g/liter, 274 mosmol) flowing at the rate of 1 ml/min. Impedance measurements were made at 18 frequencies between 5 Hz and 10 kHz at regular intervals during superfusion with sucrose. Each frequency series took ~1.5 min.

Results are expressed as specific tissue impedance, based on the observed impedance, tissue weight, tissue length (2.7 cm), and the interelectrode distance (2 cm). We assumed a specific gravity of 1.06 $g \cdot cm^{-3}$ and 35% extracellular space (Kao, 1977). No correction was made for the impedance of the measuring system because the electrodes were shown to make a negligible contribution. Measurements of the electrode impedance by substitution of electrolyte solutions for the tissue sample showed that the electrodes exhibited little frequency dependence. The impedance magnitude of sucrose with Krebs added, or of Krebs solution alone, decreased only 1.6 ± 0.36% (SD, $n = 15$) and the phase angle remained approximately zero over the frequency range from 5 Hz to 10 kHz.

Although some shunting of current through the experimental chamber and extracellular space was inevitable, the effect on our measurements was small because the resistivity of the sucrose solution was ~0.5 MΩ·cm. This was more than two orders of magnitude greater than the calculated resistivity of the tissue at short times in sucrose.

Electron Microscopy

Some of the samples of longitudinal uterine muscle were fixed for electron microscopy after completion of the length-constant experiments. Tissues were processed for thin-section electron microscopy, sectioned in transverse orientation, and quantitatively analyzed to determine gap junction frequency. The methods have been described in detail by Garfield et al. (1978) with a slight modification to improve the sensitivity of the micrograph analysis (Garfield et al., 1980).

Results are expressed as mean values ± SD. The Student's t test was used to test for differences between groups.

RESULTS

Electron Microscopy

Quantitative electron microscopic studies were performed on some of the myometrial samples that were used for physiological experiments (3–5 h after excision from the donor). The results in Table I show that tissues from delivering animals (hereafter referred to as delivering myometrium) had a large number of gap junctions. Both the frequency of gap junctions (6.75 gap junctions/1,000 μm) and the fractional area of cell membrane occupied by gap junctions (0.24%) was comparable to the values from tissues fixed *in situ* or immediately after removal from naturally delivering animals (Garfield et al., 1977, 1978). A small number of gap junctions (0.41/1,000 μm of cell membrane), occupying a small percentage of the total cell membrane (0.005%), were identified in some of the tissues from before-term animals (hereafter referred to as before-term myometrium). When individual samples were considered, the greatest incidence of gap junctions in any tissue before term (1.4/1,000 μm) was less than the smallest frequency seen at delivery (2.2/1,000 μm). These results show that gap junctions were significantly less frequent and occupied a 48-times-smaller fractional area of membrane before term than at delivery.

Cable Properties

Membrane electrical activity before term and at delivery consisted of bursts of action potentials, alternating with silent periods (Kuriyama and Csapo,

1961; Casteels and Kuriyama, 1965; Kuriyama and Suzuki, 1976; Kanda and Kuriyama, 1980; Anderson et al., 1981). Stimulating current pulses were usually applied during the quiescent phase (Fig. 1A). Membrane responses at various distances from the stimulating electrode were linear in the hyperpo-

TABLE I
GAP JUNCTIONS IN MYOMETRIUM

	n	Length of membrane	GJs	GJs/1,000 μm	Fractional area
		μm			%
Before term	8 (3)	11,373	5	0.41±0.61 ($P < 0.001$)	0.005±0.008 ($P < 0.001$)
Delivering	6 (6)	8,366	56	6.75±3.83	0.24±0.14

Frequency of gap junctions (GJs) in tissues fixed after completion of electrical recordings. *n* is the number of tissues examined; the number of tissues in which GJs were identified is in parentheses. Length of membrane refers to the total length of membrane surveyed in 20–24 micrographs of each tissue. The number of GJs is the total of all five or seven lined junctions identified and is used to calculate the frequency of GJs, expressed as the mean number of GJs/1,000 μm of nonjunctional membrane. The fractional area of GJs was calculated by doubling the total length of GJ membrane and dividing by the length of nonjunctional membrane. Values are means ± SD.

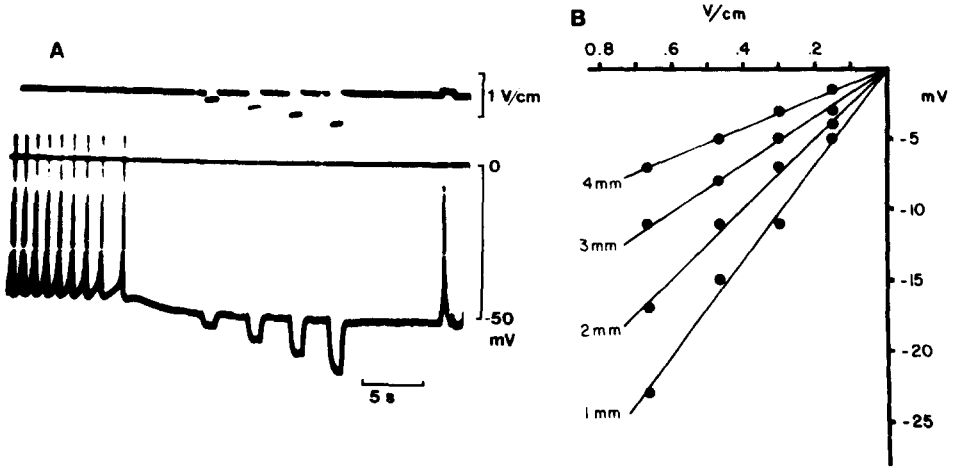


FIGURE 1. Hyperpolarizing current pulses of 1 s duration were applied to delivering myometrium, in this case, during the quiescent phase following a spontaneous burst of action potentials. A. The upper trace is the voltage gradient in the stimulating chamber, the middle trace is zero potential, and the lower sweep is the membrane response. A depolarizing stimulus at the far right evoked a single action potential. B. Current-voltage relationship shows the linear membrane responses in the hyperpolarizing direction. Steady state responses (as shown in A) were measured at four distances from the nearest stimulating electrode, as indicated in the figure.

larizing direction (Fig. 1B). Depolarizing stimuli usually initiated active membrane responses (Fig. 1A).

The resting membrane potentials of longitudinal myometrial cells were determined upon withdrawal of the microelectrode from the cells and are

summarized in Table II. The grand mean of the membrane potentials from before-term tissues was −52 ± 4.7 mV and this value fell to −46 ± 2.6 mV during delivery.

Examples of responses to hyperpolarizing current pulses are shown at various distances from the nearest stimulating electrode in Fig. 2A for before-term and delivering tissues. The amplitude of the steady state hyperpolarizing potential decayed exponentially with distance from the anode. Semilog plots of the geometric mean amplitudes ± SD at each distance are shown in Fig. 2B. The lines represent the least-squares fit to data plotted as logarithm of voltage vs. distance for all the data points. This form of data presentation and analysis was used to convey and account for the variation in the size of electrotonic potentials encountered at any given distance in individual experiments. The origin of this variability is unknown, but two possible sources are true nonuniformities of the muscle and variations in the quality of impalements.

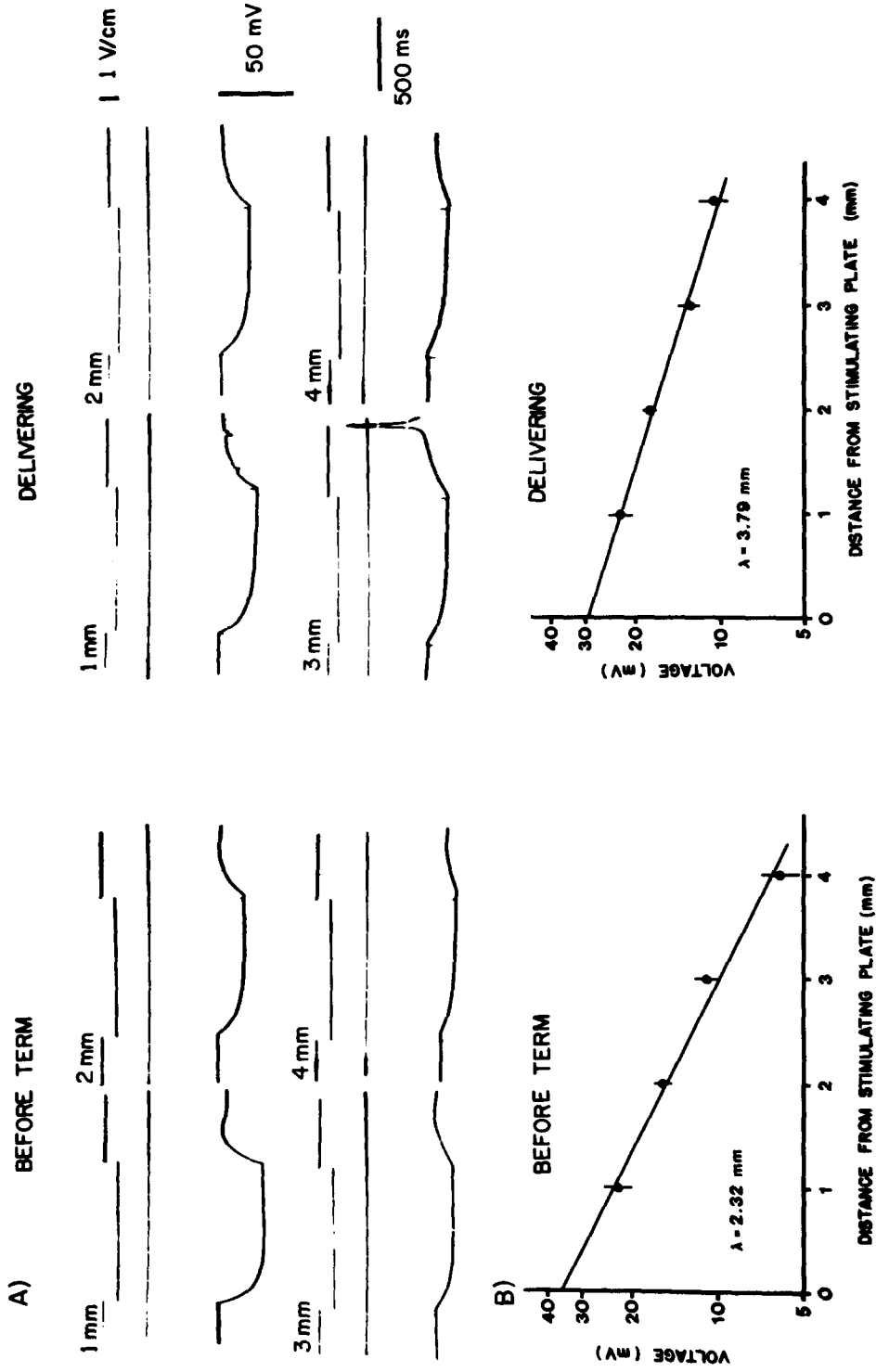
TABLE II

ELECTRICAL PARAMETERS OF MYOMETRIUM FROM BEFORE-TERM AND DELIVERING MYOMETRIUM

	Before-term	Delivering	P
	$n = 17$	$n = 16$	
Resting membrane potential (mV)	51.7±4.7	45.5±2.6	$P < 0.01$
Length constant λ (mm)	2.59±0.84	3.68±1.0	$P < 0.05$
Membrane resistance r_m (arbitrary units)	413±177	607±240	$P < 0.05$
Internal resistance r_i (arbitrary units)	64±21	43±14	$P < 0.01$
Time constant τ_m (ms)	158±61	225±72	$P < 0.05$

Summary of electrical parameters of the longitudinal myometrium from 12 animals before term and from 11 animals delivering. All values are mean ± SD. The membrane resistance and internal resistance are expressed in arbitrary units, as explained in the text.

FIGURE 2. (*opposite*) Spatial decay of hyperpolarizing potentials is shown for before-term and delivering myometrium. A. Responses are shown at four distances, 1, 2, 3, and 4 mm from the nearest stimulating electrode. B. Geometric means ± SD of the steady state amplitudes are plotted as $\log_{10}$ voltage vs. distance from the electrode. The lines were determined by least-squares regression analysis of all the data points. For these graphs there were 40 impalements before term and 33 impalements in the delivering tissue. The length constant (λ) was calculated as the distance for decay of the electrotonic potential to e^{-1}. The voltage at the anode (V_0) was determined from the intercept of the regression line with the ordinate.


A)
BEFORE TERM
DELIVERING
1 mm
2 mm
3 mm
4 mm
1 V/cm
50 mV
500 ms
B)
VOLTAGE (mV)
DISTANCE FROM STIMULATING PLATE (mm)
λ - 2.32 mm
λ - 3.79 mm

Before term, the length constants ranged from 1.7 to 4.1 mm, with a mean value of 2.6 ± 0.8 mm (Table II). Delivering tissues had length constants ranging from 2.4 to 6.1 mm and a mean value of 3.7 ± 1.0 mm (Table II). The mean value of λ was significantly larger (a 42% increase) in delivering animals.

Inasmuch as rat longitudinal myometrium exhibits cable-like properties when polarized with extracellular electrodes (Abe, 1971; Kuriyama and Suzuki, 1976; Kanda and Kuriyama, 1980; and the present findings), the equations of one-dimensional cable theory (Hodgkin and Rushton, 1946) can be applied to the tissue. Measurements of λ and the input resistance (R_0) can be used to determine values of membrane resistance (r_m) and internal resistance (r_i) (Gage and Eisenberg, 1969; Jack et al., 1975; for smooth muscle, Haeusler and Thorens, 1980).

Relative values of R_0 were obtained from the ordinate intercept (voltage at the anode, V_0) of the voltage vs. distance lines (Fig. 2B). Although no direct measure of the amount of current passed across the cell membrane in the stimulating chamber was available, we monitored the magnitude of the voltage gradient in each case. Therefore, R_0 was calculated in arbitrary units ($R_0 = V_0/I$, $mV/V \cdot cm^{-1}$). Relative values of r_m and r_i were obtained from the following relations and are given in Table II.

$$\lambda = (r_m/r_i)^{1/2} \quad \text{and} \quad R_0 = 1/2(r_m \cdot r_i)^{1/2}.$$
$$\text{Therefore, } r_m = 2R_0\lambda \quad \text{and} \quad r_i = 2R_0/\lambda.$$

During delivery, the membrane resistance was 47% larger and the internal resistance was 33% lower than before term (Table II). Both of these factors contributed to the increased λ observed in delivering tissues.

The membrane time constant (τ_m) was estimated from plots of the time to reach 50% of the steady state electrotonic potential vs. distance from the nearest stimulating electrode (Fig. 3). The slope of the line approaches $\tau_m/2\lambda$ when the length of the tissue between the stimulating electrodes is greater than $\sim 3\lambda$ (Bywater and Taylor, 1980). The values of τ_m were 158 ± 61 ms before term and 225 ± 72 ms during delivery (Table II). These values could represent underestimates of τ_m, especially in the case of delivering tissues, since $<3\lambda$ of tissue was present in the stimulating compartment in some instances.

The conduction velocity (Θ) of the half-maximal amplitude of the electrotonic potential is given by the inverse of the slope of the line in Fig. 3. Because the conduction velocity along a cable is inversely proportional to the square root of the internal resistance (Hodgkin, 1954; Jack et al., 1975), we would predict that Θ would be greater at delivery, when the internal resistance is decreased. However, in these experiments, we found that Θ was the same for both categories of muscle, 3.4 ± 0.5 $cm \cdot s^{-1}$ (n = 10 before term, n = 14 at delivery). Simultaneous changes in one or more of the other parameters that determine Θ (e.g., an increase in membrane resistance) may have concealed any change caused by a decrease in internal resistance.

Longitudinal Impedance of Myometrium

The longitudinal impedance of myometrium was measured to determine whether the decrease in internal resistance of the tissue was caused by a

change in the myoplasmic or junctional components. We assumed that, with the methods used, uniform polarization of the preparation was achieved. Fig. 4 illustrates the longitudinal impedance characteristics of myometrium, obtained 5 min after the flow of sucrose began. The impedance magnitude exhibited a marked dependence on the frequency of the alternating current, decreasing with increasing frequency. At the low- and high-frequency extremes, the magnitude was relatively independent of frequency. The phase angle (ϕ) of the impedance was always negative, that is, capacitive, and showed a single peak in before-term and postpartum myometrium. The frequency response of myometrium was displayed on the real (R)–imaginary (X) plane, according to the relationship $Z = (R^2 + X^2)^{1/2}$ and $\tan \phi = X/R$. The frequency dispersion of impedance values plotted in this fashion described arcs of circles for tissues from animals before term (Fig. 4A) and postpartum

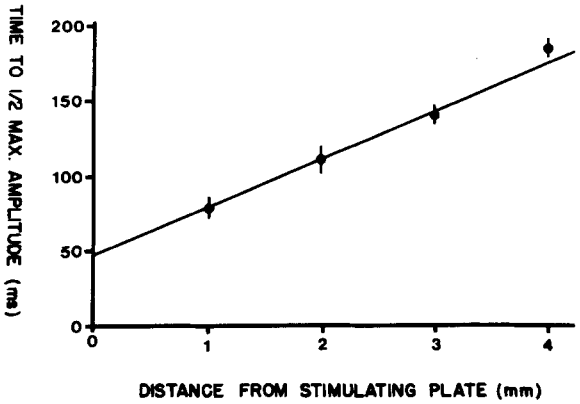


FIGURE 3. Graphs of time to reach half-maximum amplitude vs. distance from the nearest stimulating electrode were used to estimate the membrane time constant (τ_m) and conduction velocity (Θ). Circles are mean values ± SD of several measurements at each distance, in this case for delivering myometrium. The line was fitted by least-squares regression analysis; the slope was used to calculate τ_m and Θ (see text). $\tau = 2\lambda/\text{slope} = 248$ ms.

(Fig. 4C), which strongly supports our assumption of uniform tissue polarization. The centers of the circles were always depressed below the real axis. At frequency extremes, the impedance was essentially resistive, there being little capacitive reactance. Impedance loci could not be fitted to the frequency response of delivering tissues (Fig. 4B).

These results show that there exists in myometrium a frequency-dependent component, a capacitance, in the longitudinal direction. Studies of several smooth muscle types (Tomita, 1969; Goto et al., 1976; Ohba et al., 1976; Bortoff and Gilloteaux, 1980) and of cardiac tissues (Sperelakis and Hoshiko, 1961; Freygang and Trautwein, 1970; Stibitz and McCann, 1974; Chapman and Fry, 1978) have provided evidence for a capacitance oriented in the longitudinal direction. Because the cytoplasm of cells is purely resistive (nerve: Cole and Hodgkin, 1939; skeletal muscle fiber: Mobley et al., 1975; Caillé,

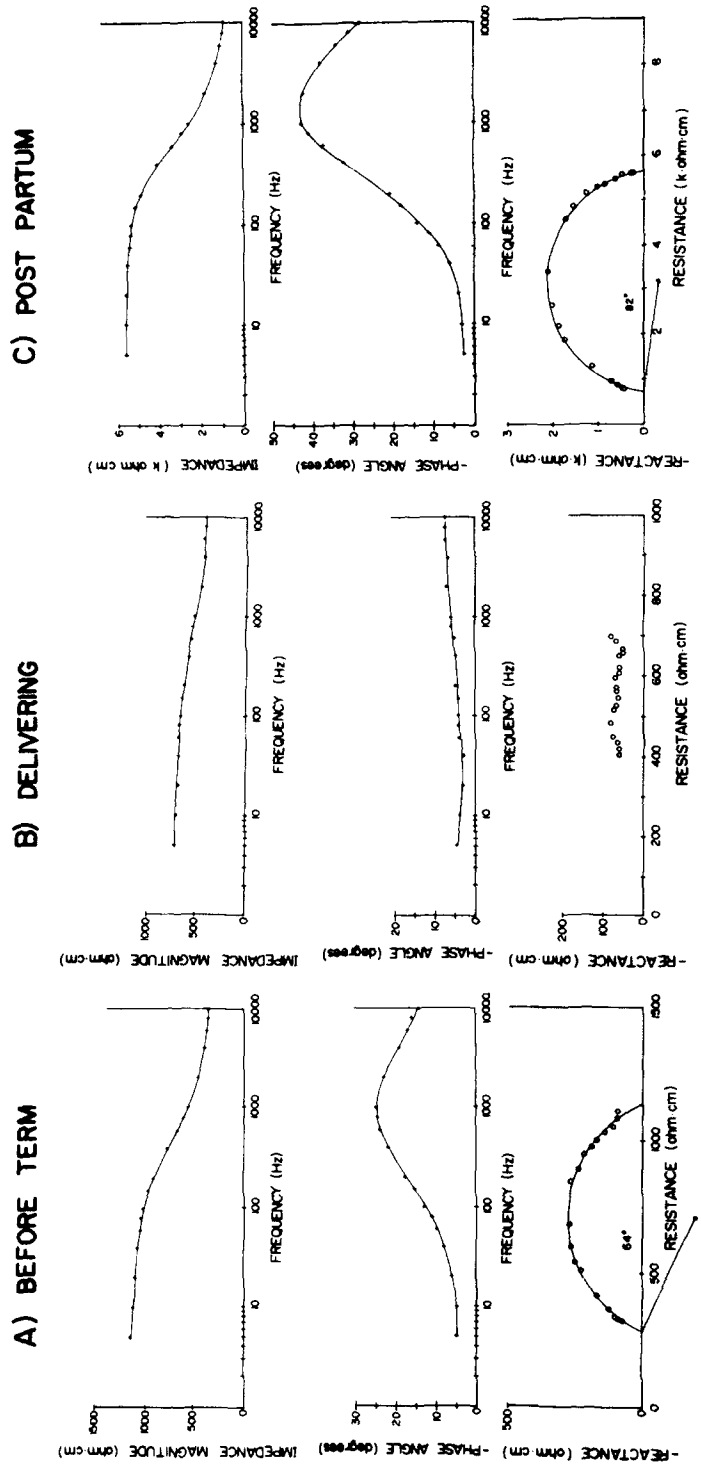


FIGURE 4. Impedance responses of before-term (A), delivering (B), and postpartum (C) myometrium at 5 min in sucrose. Impedance magnitude decreased with increasing frequency and phase angles were negative over the frequency range, indicating the presence of a capacitance oriented in the longitudinal direction. Impedance magnitude and phase for each frequency were plotted as impedance loci (see Methods), with the frequency increasing counterclockwise.

1975), it is reasonable to assume that the capacitance is associated with current pathways, i.e., junctions, between cells.

Equivalent Circuit

An equivalent circuit has been proposed by Tomita (1969) to account for the impedance properties of smooth muscle when current is constrained to flow in the longitudinal axis. As shown in Fig. 5, the model describes the junctional resistance between cells as being in parallel with the capacity component of

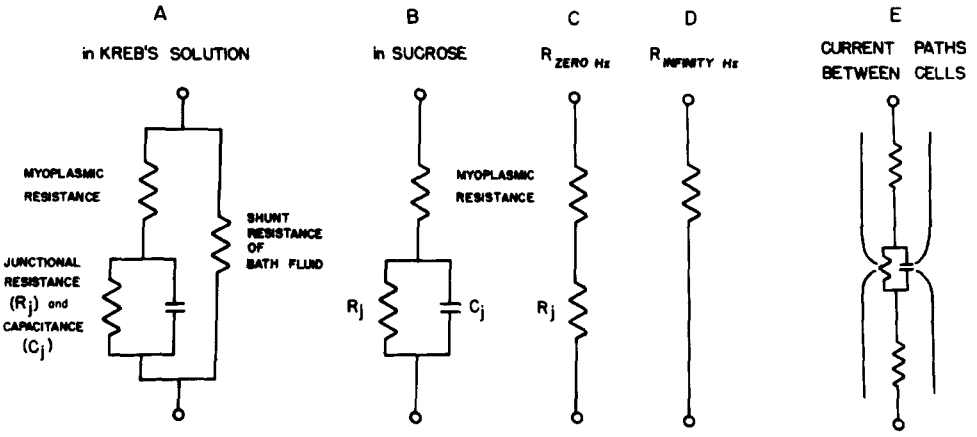


FIGURE 5. Model equivalent circuit of smooth muscle under various conditions (after Tomita [1969] and Ohba et al. [1976]). A. Total impedance of tissue with shunt resistance of extracellular medium. R_j and C_j are junctional resistance and capacitance. B. Tissue impedance after washout of extracellular medium with sucrose. C. Tissue resistance at 0 Hz, $R_{0\ Hz}$, is the sum of the myoplasmic and junctional components. D. $R_{\infty\ Hz}$ represents myoplasmic impedance. E. Model of the current pathways between smooth muscle cells.

the junction and the surrounding cell membrane, all in series with the resistance of the myoplasm. According to the model, the junctional impedance would decrease with increasing frequency due to a bypass of current through the capacitance. Therefore, the impedance measured at high frequencies would probably be caused solely by the resistance of the myoplasm, whereas the impedance that disappears with increasing frequency could be attributed to the junctional membrane. Despite some deficiencies in this model (see Discussion), we have chosen to use it to interpret the impedance response of myometrium and compare the quantitative aspects of several types of myometrium. Extrapolation of the low-frequency impedance magnitude values, plotted on a linear frequency scale, gave values of the impedance at 0 Hz, $R_{0\ Hz}$. This parameter corresponds to the internal (core) resistance in the steady state, R_i. It is composed of the resistance of the myoplasm and the

junctions. Because the impedance magnitude was nearly independent of frequency at high frequencies, the 10-kHz value of the impedance was used as an estimate of $R_{\infty\ Hz}$, the myoplasmic resistance. The difference between 0 Hz and 10 kHz was assumed to represent the junctional resistance, R_j.

Changes in the Impedance Caused by Isosmotic Sucrose

The myometrium was superfused with isosmotic sucrose to reduce the shunting of current through the tissue chamber and extracellular space. As a result, the tissue impedance increased with time, although the form of the response was unchanged (see also Tomita, 1969; Ohba et al., 1976). 5 min is likely to be sufficient time for washout of ions from the extracellular space of rat myometrium (Hamon et al., 1976), so the increase in impedance that is seen after 5 min may be caused in part by the leaching of ions from the intracellular compartments. To correct for this change, we analyzed the results according to the methods used by Ohba et al. (1976). Conductivity values (resistivity^{-1}) were plotted as a function of time in sucrose (Fig. 6) and estimates of the conductivity before exposure to sucrose (i.e., time zero) were obtained by extrapolating the curves to the ordinate. This form of presentation was used (Ohba et al., 1976) because of the similarities between the decay of conductivity (presumably caused by washout of ions) and the fluxes of radioisotopes from smooth muscle (for example, see Hamon et al., 1976). In both instances, the changes have been shown to consist of several exponential processes.

We are aware that the need to extrapolate our results over time is a drawback of this experimental technique. Because of this step our results can only be viewed as rough estimates of the true resistivity. But the extrapolation procedure does not appear to contribute to the differences between groups, so the comparative aspects of this study are not compromised.

Our estimates of the electrical parameters are summarized in Table III. The total tissue resistance ($R_{0\ Hz}$) decreased from before term to delivery. This change occurred in the presence of constant myoplasmic resistivity, 319 ± 113 Ω·cm before term and 340 ± 93 Ω·cm at delivery. The decrease in total tissue resistance was attributable to a decrease in the junctional component, from 323 ± 161 Ω·cm before term to 134 ± 64 Ω·cm ($P < 0.01$) in delivering tissues. Both myoplasmic and junctional resistance values were larger in postpartum tissues.

Extrapolation of the conductivity values to zero time (Fig. 6) gave estimates of tissue resistivity that were ~25% lower than the values recorded at 5 min in sucrose (time of the first measurements). However, the extrapolation procedure itself did not contribute to the differences observed between tissues from before-term, delivering, and postpartum animals. This is evident from two observations. First, at 5 min the myoplasmic resistivity values were the same before term and at delivery, but the junctional resistivity values were significantly lower at delivery ($P < 0.001$). Junctional and myoplasmic resistivity was higher postpartum. This is the same pattern that was shown in Table III.

Second, analysis of the conductivity vs. time curves by curve-peeling techniques showed that the rate of change of the conductivity was about the same for both before-term and delivering tissues (results not shown).

The time constant of the junction was determined according to the relationship $\tau_j = 1/2\pi f_0$, where the characteristic frequency (f_0) was the frequency

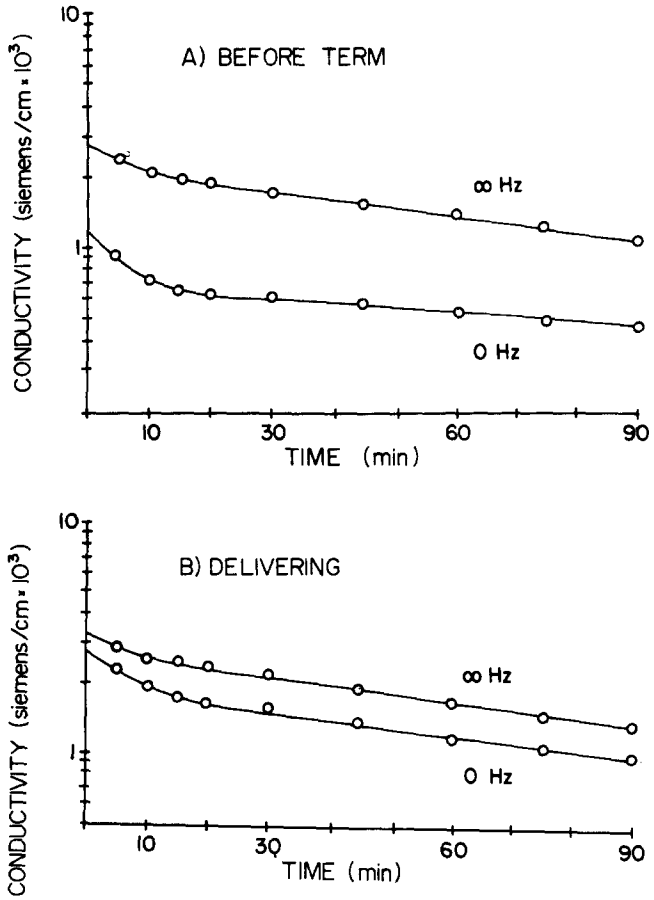


FIGURE 6. Changes in conductivity (resistivity^{-1}) of before-term (A) and delivering (B) myometrium with time in sucrose. Specific resistance values were determined by extrapolating lines to the ordinate.

with the maximum reactance. The τ_j remained relatively constant in all types of tissues at ~0.2–0.3 ms, values that are <1% of the membrane time constant (Table II).

Method of Standardizing the Results

We have standardized all of our results to units of specific resistance using the individual sample weights as a measure of the effective cross-sectional area of

conducting tissue. If the weight of the samples is a good measure of the cross-sectional area and provides a valid means of correcting for variations in sample size, we would predict that the following relation would hold true:

$$Y = \text{weight} \cdot K/Z(t)$$

Where Y is the measured admittance, impedance^{-1}, units siemens; weight is the weight of the muscle sample in grams; K is a constant comprised of the cellular space, tissue length, interelectrode distance, and density. The admittance should be directly related to the tissue weight and inversely proportional to the true specific impedance of the tissue, $Z(t)$. The distributions of data points were roughly linear, in agreement with our prediction and could be fitted by lines (Fig. 7). However, the least-squares lines did not pass through

TABLE III
SPECIFIC RESISTANCE OF MYOMETRIUM DURING PREGNANCY

	Total tissue resistance ($R_{0\,Hz}$)	Myoplasmic resistance ($R_{\infty\,Hz}$)	Junctional resistance (R_j)
17–22 days gestation ($n = 18$)	642±220 ($P < 0.05$)	319±113 ($P > 0.05$)	323±161 ($P < 0.01$)
Delivering ($n = 13$)	474±126	340±93	134±64
Postpartum 1.5–2 d ($n = 7$)	2,120±924	756±196	1,364±940

Values of specific resistance (Ω·cm) are means ± SD. n = number of tissues examined, from eight animals before term, seven animals delivering, and four animals postpartum. All values were determined from the impedance magnitude, and junctional resistance was determined by $R_{0\,Hz} - R_{\infty\,Hz}$. Resistance of postpartum myometrium was significantly greater than the other categories in all cases ($P < 0.01$). P values indicate levels of confidence between before-term and delivering samples.

the origin, which might indicate errors in the measurements of the tissue impedance or weight. At 10 kHz (Fig. 7A), data from before-term and delivering tissues overlap and the slopes of regression lines are about the same. Thus, the specific impedance of the myoplasm is the same before term and at delivery, as described in Table III. The admittance at 5 Hz (Fig. 7B) reflects the difference between the total tissue impedance of before-term and delivering tissues. Other factors being equal, the steeper slope for delivering tissues is evidence for a smaller specific impedance. Thus, without any correction factors, the "raw data" illustrate the different properties of before-term and delivering tissues. The difference between the specific impedances predicted from the slopes is slightly greater than the difference at time zero presented in Table III.

DISCUSSION

Our aim has been to investigate the significance of gap junction formation in the myometrium at parturition. In this report we have established a temporal relationship between a change in structure and a change in the passive electrical properties of the myometrium. The results are consistent with the view that increased numbers of gap junctions between smooth muscle cells facilitate the flow of current in the parturient uterus.

Ultrastructural studies of some of the same samples used for in vitro physiological experiments confirmed that gap junctions were present in all delivering tissues examined after the cable studies (Table I). A small number of gap junctions could be identified in some of the tissues from animals 20–22

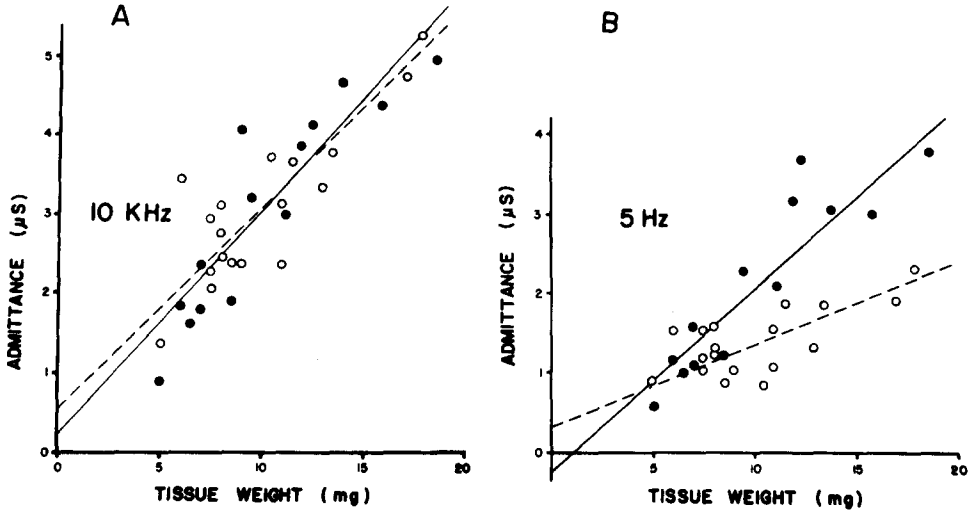


FIGURE 7. Linear relationship between sample weight and admittance (impedance^{-1}) at 5 min in sucrose. Open circles and broken lines represent before-term tissues. Closed circles and solid lines represent delivering tissues. Lines were fitted by least-squares method. A. Admittance at 10 kHz, the slopes of the two tissue types were similar. B. At 5 Hz the admittance vs. weight line was steeper for delivering samples, which suggests that the specific resistance of the tissue was smaller (see text).

d pregnant but not delivering. The mean values of gap junction frequency and fractional area were significantly different in the two groups. When individual samples were considered, the groups were non-overlapping. Thus, evidence shows that we have studied distinct groups of myometrium that were different in regard to the incidence of gap junctions. Moreover, if we assume that the gap junctions we observe are functional and are the basis of the physiological change at term, then the presence of gap junctions before term should serve only to conceal, and not cause, the differences between the two categories of muscle. The fact that we were able to discern differences at delivery suggests that the groups were functionally distinct. We did not

examine the ultrastructure of all muscle used in the physiology experiments and assume that our sampling was representative of tissues in both the cable and impedance experiments. We also assume, from earlier studies (Garfield et al., 1978), that few or no gap junctions were present in tissues from postpartum animals.

There are two possible explanations for the presence of gap junctions in our tissues before term. We may have selected animals in which in vivo formation of gap junctions preceded identifiable labor. Alternatively, some gap junctions may have formed during the course of the in vitro experiments (Garfield et al., 1978).

We cannot exclude entirely the possibility that changes in the myometrium at the end of gestation other than gap junction formation (e.g., changes in cell geometry) contribute to the observed differences. However, we have reduced the likelihood of this happening, and have made the best comparison possible, by studying myometrium of 17–22-d-pregnant, delivering, and shortly postpartum animals. Growth of the myometrium (Afting and Elce, 1978) and the fetuses (Knox and Lister-Rosenoer, 1978) are both reduced at the end of term. Therefore, it is unlikely that any significant change in myometrial structure took place at the end of term associated with uterine or fetal growth.

Changes in the Electrical Characteristics

Several differences in the electrical characteristics of before-term and delivering myometrium were apparent. The resting membrane potential decreased from before term to delivery, consistent with previous reports on rat myometrium (Casteels and Kuriyama, 1965; Kuriyama and Suzuki, 1976; Kanda and Kuriyama, 1980; Anderson et al., 1981). The mean values of λ we obtained (Table II) were larger than that reported by Abe (1971) for mid- and late-term myometrium (1.8 mm). Kuriyama and Suzuki (1976) grouped before-term with delivering tissues and reported a mean λ of 2.9 mm, close to the value we found for before-term animals alone. They also reported that a small sample ($n = 3$) of delivering tissues had a λ of 3.7 mm (see Fig. 9, Kuriyama and Suzuki, 1976). Bearing in mind that tissues at the end of gestation must be properly categorized to account for changes caused by gap junction formation, these values are very similar to ours.

Our results differ from those of Zelcer and Daniel (1979), who did not identify any change in λ at parturition. We believe that the values reported in this study accurately reflect the changes that occur at parturition. Several factors may account for the contradictory results. We used longer and wider muscle samples aligned so that the decay of potentials occurred in the ovarian-cervical direction. In addition, we consistently maintained the samples at their in vivo, distended length (see Methods). We have not systematically analyzed the effect of the first two factors, but the amount of stretch imposed on samples of myometrium did influence the measured cable parameters (unpublished observations). Because of the experimental protocol used to ensure consistent alignment and stretch, these variables did not enter into our experiments, allowing us, we believe, to resolve the increase in λ at parturition.

The degree of stretch placed on smooth muscle tissues in vitro is an important variable that has not always been adequately acknowledged and controlled for in studies of cable properties. The possibility has been raised that variations in stretch may have contributed to the large scatter in values of λ reported for various smooth muscle (Tomita, 1975). Our procedure for maintaining the muscle at its in vivo length during experiments may not be universally applicable (for example, if a particular smooth muscle exhibits marked variation in its length in vivo), but it is an attempt to deal with this problem systematically.

The λ in pregnant myometrium is many times the length of each cell (300–600 μm), as is the case for many smooth muscle tissues (Tomita, 1975) and single smooth muscle cells (Singer and Walsh, 1980). The relatively good coupling before term existed when gap junctions were either present in low frequency or were absent. In view of the widely held belief that the gap junction is the morphological correlate of low-resistance coupling (Bennett and Goodenough, 1978; Loewenstein, 1981), the basis of the coupling before term is uncertain. Perhaps only a very low area of gap junction contact between smooth muscle cells is required for coupling. The increased frequency at parturition would serve, as we have suggested, to further reduce the junctional resistance between cells. Alternatively, coupling could occur in the absence of visible gap junctions. Small gap junctions, undetectable by thin-section electron microscopy, may exist (see Williams and DeHaan, 1981), or other morphological structures may provide low-resistance pathways for current flow between cells (Daniel et al., 1976). Whether gap junctions are necessary for cell coupling is a subject of debate, but available evidence, including results described in this investigation, suggests that the junctions are sufficient for coupling.

The length constant has been defined as $\lambda = (r_m/r_i)^{1/2}$, or if we consider the specific cable parameters, $\lambda = (R_m a/R_i 2)^{1/2}$, where $r_m = R_m/2\pi a$, $r_i = R_i/\pi a^2$. The cable radius (a) is the radius of the individual smooth muscle fibers. An increase in the specific membrane resistance, a decrease in the specific internal resistance, or increased cable radius could be the cause of the change in λ observed at term. We have assumed that the fiber radius is constant before term and at delivery, having no reason to believe that cells of the myometrium undergo any significant growth at the end of pregnancy (Afting and Elce, 1978). (If any change at all is occurring, the cells are being stretched further by the growth of the fetuses, so the cable radius should decrease. This would cause a change opposite to the one we have observed.)

The increase in λ at delivery can be attributed in part to an increase in membrane resistance (Table II). Consistent with our observation at delivery, several workers have reported that membrane depolarization in myometrium is accompanied by an increase in membrane resistance (Bülbring and Kuriyama, 1973; Kuriyama and Suzuki, 1976). This is possibly related to decreased potassium conductance (Tomita and Watanabe, 1973). We observed membrane time constants that were similar to those reported by Kuriyama and Suzuki (1976). The larger τ_m at delivery (Table II) was probably the result of

the increased membrane resistance. Both parameters increased by the same amount (~45%) from before term to delivery.

The second factor that contributed to the change in λ at delivery was the 33% decrease in internal resistance (per unit length) from before term to delivery (Table II). This observation was verified by the impedance studies, which showed a 26% decrease in longitudinal impedance (taken to represent internal resistance) from before term to delivery. (Relative changes of the two units of internal resistance are directly comparable, if we assume that the cable radius is constant.) Thus, two independent experimental methods for assessing the internal resistance of myometrium yielded similar results. The internal resistance of myometrium is composed of the resistance of the myoplasm of individual cells plus the resistance of junctions between cells. Results from the impedance analysis indicate that the myoplasmic resistance was constant and that the junctional resistance decreased by ~60% from before term to delivery. With this evidence to support the (usual) assumption of constant cytoplasmic resistivity, we interpret the decrease in internal resistance measured by cable analysis also to reflect decreased junctional resistance between muscle cells. 1.5–2 d after delivery, a time when few, if any, gap junctions remain in the myometrium, the junctional as well as the myoplasmic resistance was larger (Table III).

Therefore, our results provide electrophysiological evidence for closer coupling of myometrial cells during delivery. Taken together with ultrastructural evidence, these data support, but do not prove, our hypothesis that gap junction formation causes an improvement in electrical coupling in parturient myometrium.

If a fiber radius of 5 μm is assumed, the values of specific internal resistance (from the impedance studies) and λ can be used to derive estimates of specific membrane resistance (R_m). The membrane time constant is given by the relation $\tau_m = R_m C_m$, so estimates of membrane capacitance were also made. Before-term R_m was 87 k$\Omega \cdot$cm^2 and C_m was 1.8 μF/cm^2. At delivery R_m was 130 k$\Omega \cdot$cm^2 and C_m was 1.7 μF/cm^2. These values are subject to considerable error, but are within the range found for a variety of smooth muscle tissues (Tomita, 1975) and single muscle cells (Singer and Walsh, 1980).

Effects of Isosmotic Sucrose

The strips of muscle used in the impedance experiments were superfused with ion-free, isosmotic sucrose. This step was necessary to reduce the shunting of current in the extracellular space and constrain the current flow through the muscle primarily to the longitudinal direction. This procedure has the drawback of causing the measured impedance to increase with time, as shown in Fig. 6.

Uncoupling of cardiac muscle cells, indicated by an increased internal resistance, is thought to occur after 15–60 min in Ca^{++}-free solutions (New and Trautwein, 1972; Kléber, 1973). However, the longitudinal impedance of intestinal smooth muscle was insensitive to the external Ca^{++} concentration (Tomita, 1969). We estimate that the concentration of Ca^{++} in the sucrose

was <1 μM. Functional and structural uncoupling of uterine smooth muscle cells may have occurred in our experiments and contributed to the increase in specific resistance that was observed, but the form of the frequency responses did not change with time. The extrapolation to time zero should reduce the likelihood of this factor influencing our estimates of tissue resistance to any great extent. If Ca^{++}-free solutions cause closure of gap junctional channels in myometrium, parturient muscle should be most susceptible to this effect. Therefore, it is unlikely that uncoupling could explain the decrease in junctional resistance that we observed at parturition.

Assumed Equivalent Circuit

We have assumed from the outset that the equivalent circuit described in Fig. 5 (after Tomita, 1969) was adequate for representing myometrium. This circuit is an oversimplification and some differences from the predicted frequency responses were evident, especially in myometrium from before-term and delivering animals. The phase responses were relatively flat and the centers of the impedance loci were depressed far below the real axes (before term) or loci were nonexistent (delivering). For quantitative comparisons of delivering myometrium to other stages, we have relied upon the impedance magnitude values, so these deviations from the expected responses should not affect our interpretation of the results. The basis of the depressed center of the impedance locus is not known, although the presence of a constant phase shift element or inhomogeneity of the sample, resulting in multiple time constants, have been suggested as causes (Cole, 1968; Schanne and Ceretti, 1978). The equivalent circuit may not be appropriate for delivering myometrium, which exhibited none of the predicted characteristics other than decreased impedance magnitude with increased frequency. Such a nonspecific change would be expected of any linear network composed of resistors and capacitors and offers no information about the arrangement of the circuit elements involved. It is unclear how a quantitative change in the number of gap junctions could lead to qualitative alteration in the equivalent circuit. We are investigating possible causes of the depressed loci and more appropriate circuit models for myometrium.

Regardless of the uncertainties associated with the equivalent circuit, our values of the impedance at 0 Hz are good estimates of the total specific resistance or effective resistivity of myometrium. The decrease that we observed at parturition supports the hypothesis that the internal resistance of myometrium is lower at delivery. But, of course, without the additional information obtained from measurements of resistance at high frequencies, we would not have been able to distinguish between a decrease in the resistance of junctions or myoplasm.

Role of Gap Junctions in Regulation of Muscle Activity

These results suggest that improved coupling of myometrial cells is one factor involved in the conversion of the uterus from a relatively quiescent organ to one that expels the uterine contents in an orderly manner. Several reports

have correlated changes in the mechanical activity of muscle with alterations in the electrical properties. Some normally quiescent smooth muscles respond to treatment with tetraethylammonium ion by developing phasic contractions, representing the synchronous mechanical activity of many muscle cells. This transition has been associated with increased λ (Kroeger and Stephens, 1975), increased membrane resistance, and decreased internal resistance (Haeusler and Thorens, 1980). The latter change could be related to an increased incidence of gap junctions between smooth muscle cells (Kannan and Daniel, 1978).

Denervation-induced phasic activity of vas deferens is accompanied by an increased λ in some tissues (Goto et al., 1978), which may be related to a decrease in the junctional resistance (Goto et al., 1976). It is uncertain whether modulation of gap junctions occurs in this particular experimental model, because some investigators were unable to identify any gap junctions between the smooth muscle cells of either intact or chemically denervated vas deferens (Paton et al., 1976).

The onset of synchronous beating of heart cell aggregates has been correlated with a decrease in coupling resistance, which is thought to be associated with increased area of gap junction contact between the cells (Clapham et al., 1980). It is apparent, therefore, that regulation of electrical and mechanical activity of several muscle syncytia, including myometrium, can be achieved by modulation of junctional communication.

Administration of estrogens to nonpregnant animals results in growth of the myometrium, synchronization of myometrial electrical activity, and an increase in the length constant of the tissues (Kuriyama and Suzuki, 1976; Kao, 1977). A decrease in the junctional resistance between myometrial cells could be the basis of some of the changes in electrical properties (Bortoff and Gilloteaux, 1980). Estrogens are thought to stimulate gap junction formation in the myometrium at parturition (Garfield et al., 1980). Increased estrogen and decreased progesterone levels at the end of pregnancy in the rat (Thorburn and Challis, 1979) may contribute to the improved coupling that we observed at parturition. It should also be noted that large doses of synthetic estrogen can induce gap junction formation in nonpregnant myometrium (Dahl and Berger, 1978; Merk et al., 1980; MacKenzie and Garfield, unpublished observations). As yet there is no direct evidence to suggest that the hormonal treatments used by other workers to modify the electrical properties influenced the occurrence of gap junctions in the myometrium.

Not only the longitudinal, but the circular muscle layers of the rat uterus exhibit the characteristic transition of electrical and mechanical activity that culminates in normal parturition (Anderson et al., 1981). Gap junctions appear at parturition in both muscle layers in approximately equal numbers (Garfield et al., 1977, 1978). Furthermore, in all other species studied to date, increased numbers of gap junctions between myometrial cells accompany the evolution of coordinated activity at labor. This includes mice (Dahl and Berger, 1978), guinea pigs, sheep, and humans (Garfield et al., 1979). We can only speculate that electrophysiological changes of the sort described here

accompany the structural changes in these other instances. The widespread occurrence of gap junction formation at parturition suggests that this mechanism of regulating uterine activity may be of general significance.

Finally, it is worth emphasizing that many factors are involved in the initiation and progression of labor (Liggins, 1979; Thorburn and Challis, 1979). Hormones and prostaglandins act directly on smooth muscle cells to regulate excitability and contractility. Improved ionic coupling resulting from gap junction formation represents just one mechanism that may operate to ensure synchronous activation of the myometrium and safe, effective delivery of the young.

We thank Ms. D. Merrett for technical assistance and G. Wilson for the use of plotting programs.

This study was supported by the Medical Research Council (Canada), who also supported S. Sims with a Studentship.

Received for publication 13 November 1981 and in revised form 24 May 1982.

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